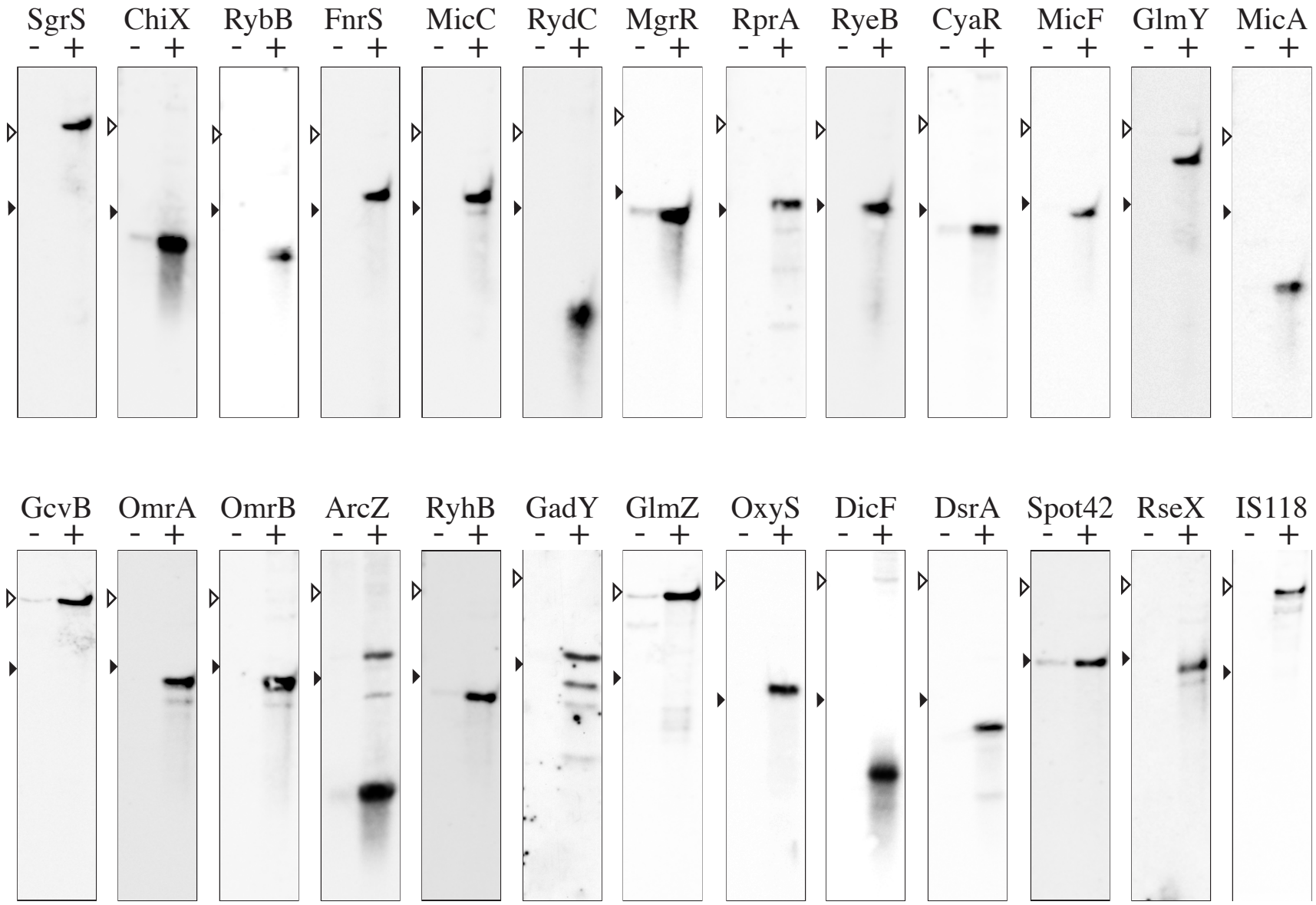
Supplementary Figures S1-S7
Supplementary Tables1-2
Supplementary Materials and Methods

Supplementary Figures:

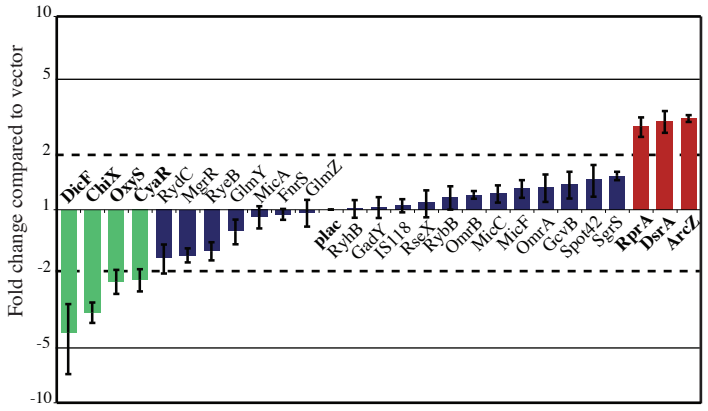
Figure S1. Northern blot on the sRNAs overexpressed from the plasmid library. Northern blots were performed on RNA extracts from the NM525 strain (carrying a *lacI*^{q} allele to allow better repression of sRNA expression from the plasmid in absence of inducer) containing either the vector control pBRplac (-) or a plasmid encoding the sRNAs listed in Table I (+). Cells were grown at 37ºC in LB containing ampicillin (100 μg/mL) and IPTG (100μM) to an OD_{600}=0.5 before samples were taken and RNA prepared. Northern blots were performed as described in Material and Methods and the sRNAs were detected using oligonucleotide probes described in table S2. Note that when the sRNA could be detected from the chromosome in the vector control lane (see ChiX, MgrR, CyaR, GcvB, GlmZ, and Spot42), it migrated at the same position as that seen from the plasmid. The filled and open triangles show the 100 nt and 200 nt positions, respectively, as determined using a century marker RNA ladder (Ambion).


SgrS
ChiX
RybB
FnrS
MicC
RydC
MgrR
RprA
RyeB
CyaR
MicF
GlmY
MicA
GcvB
OmrA
OmrB
ArcZ
RyhB
GadY
GlmZ
OxyS
DicF
DsrA
Spot42
RseX
IS118
- +

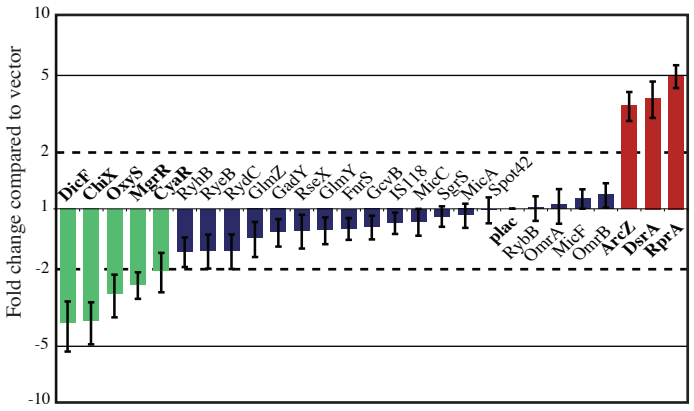
Fig_S1

Figure S2. Effect of the sRNA library on the *rpoS-lacZ* fusion

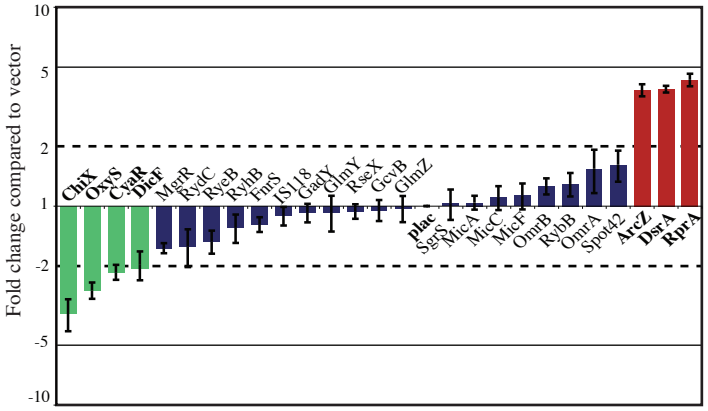
The strain PM1409, or its isogenic derivatives deleted either for *dsrA* (PM1411), *rprA* (PM1412), *arcZ* (PM1413), or carrying a triple mutations in each of the three sRNAs (PM1417) were transformed with either the pBR-plac control plasmid or with plasmids containing the sRNAs described in Table I. Transformed cells were grown in microtiter plates as in Fig. 1. The effect of the overexpression of each sRNA on the *rpoS-lacZ* fusion was plotted as a function of the fold change it induced compared to the basal activity of the strain containing the pBR-plac control vector. Fold changes greater than 2 (shown by dotted lines) were considered significant. Blue bars represent sRNAs for which effects were not considered significant; green and red bars indicate sRNAs having an activating or a repressing effect, respectively.



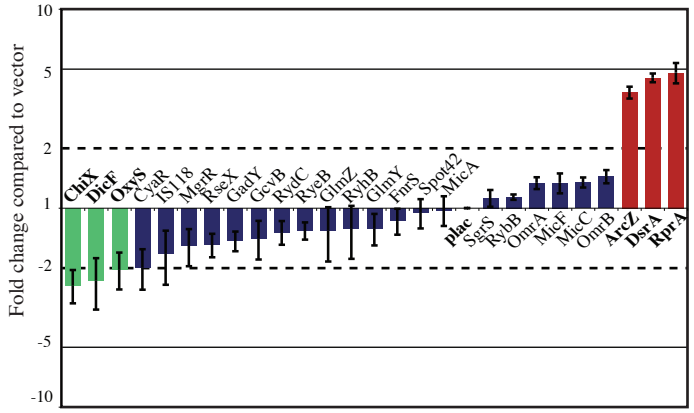
PBAD-*rpoS-lacZ* WT



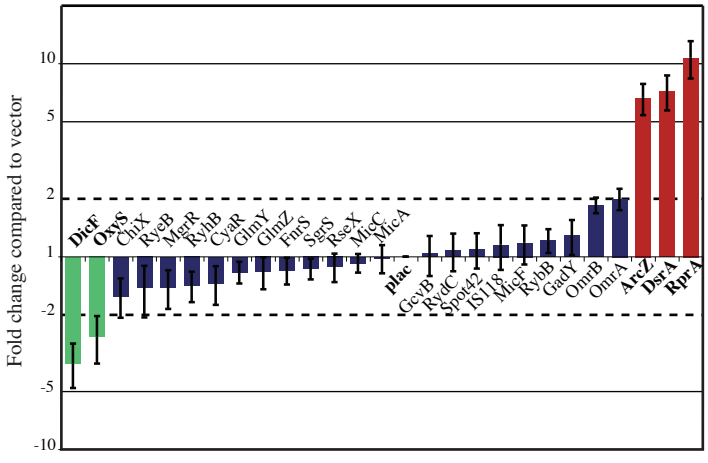
PBAD-*rpoS-lacZ* Δ*dsrA*



PBAD-*rpoS-lacZ* Δ*rprA*



PBAD-*rpoS-lacZ* Δ*arcZ*

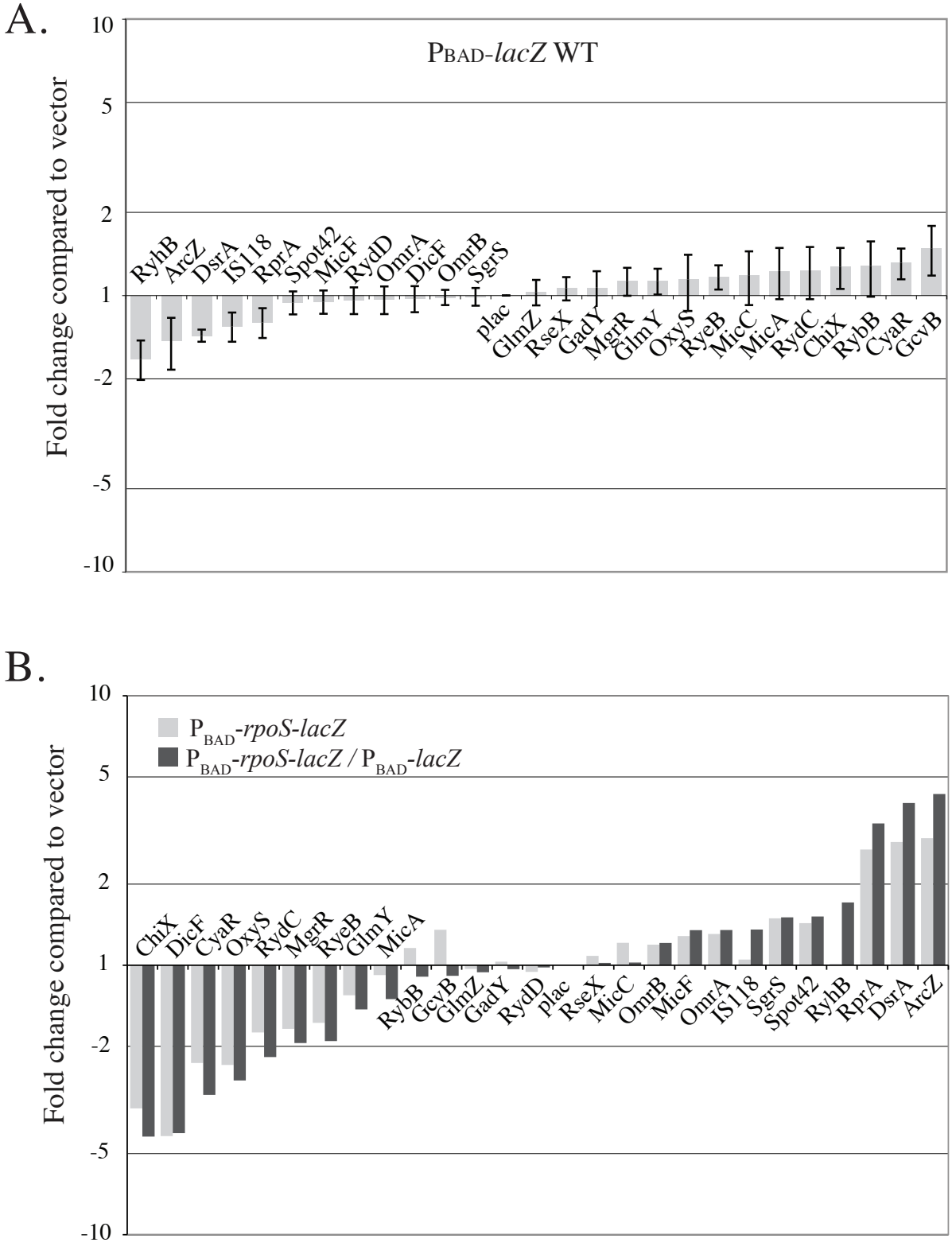


PBAD-*rpoS-lacZ* Δ*dsrA*, Δ*rprA*, Δ*arcZ*

Fig_S2

Figure S3. Effect of the sRNA library on a control P_{BAD}-*lacZ* fusion

A. Experiments are as for Fig. 1 and Fig. S2, except that the strain used was PM1051, NM525 carrying a P_{BAD}-*lacZ* fusion. The effect of the overexpression of each sRNA on the P_{BAD}-*lacZ* fusion was plotted as a function of the fold change it induced compared to the basal activity of strain PM1051 containing the pBR-plac control vector. Note that none of the plasmids has an effect greater than +/- 2 fold. B. sRNA library screening of the *rpos-lacZ* fusion normalized to P_{BAD}-*lacZ* fusion. Light grey bars: effect of sRNA expression on the P_{BAD}-*rpoS-lacZ* fusion (see figure 1) before normalization. Dark grey bars: normalized values obtained by dividing the fold activation/repression by the values obtained with the control P_{BAD}-*lacZ* fusion.

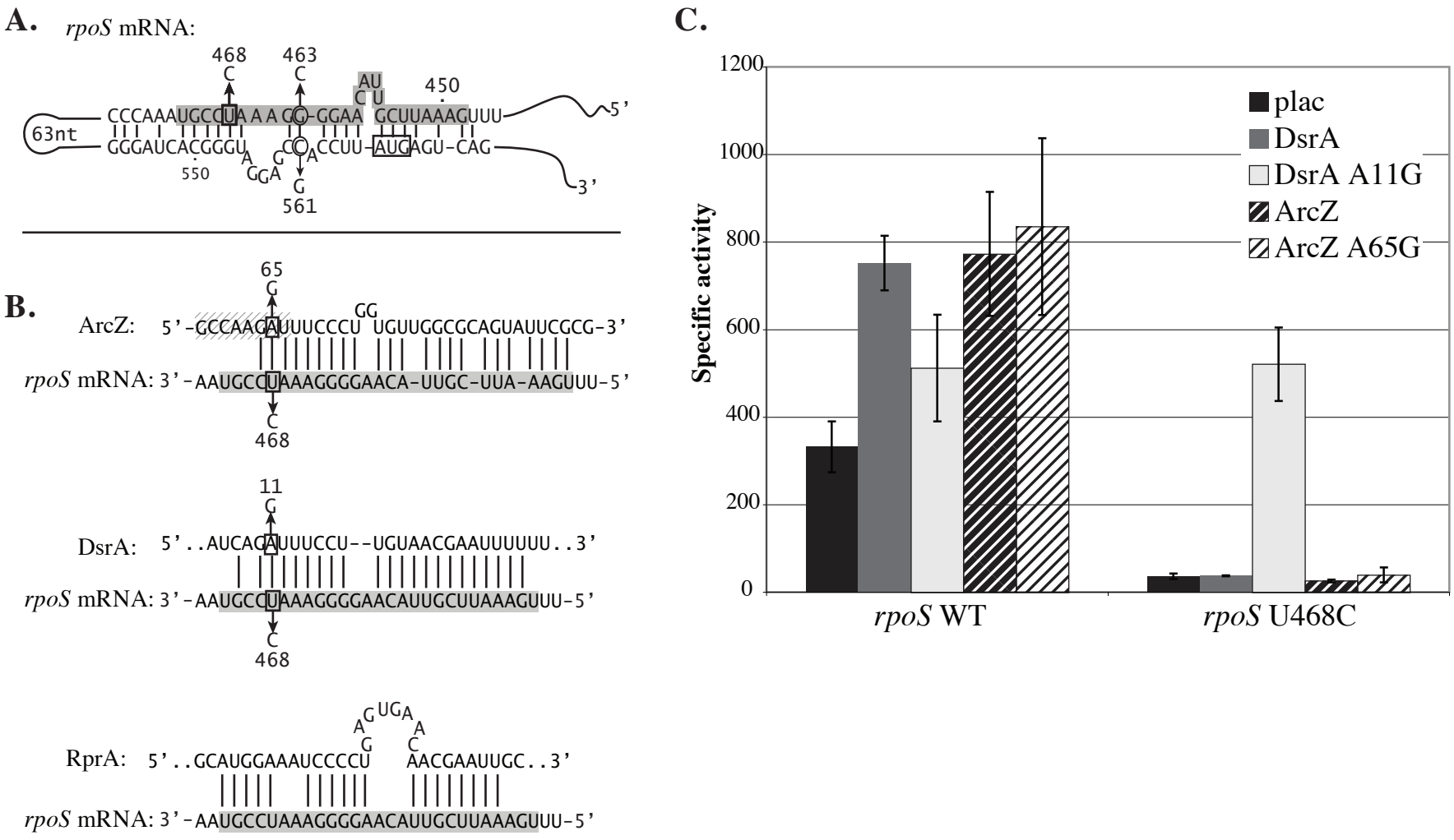

A.
PBAD-lacZ WT
Fold change compared to vector
10
5
2
1
-2
-5
-10
RyhB
ArcZ
DsrA
IS118
RprA
Spot42
MicF
RydD
OmrA
DicF
OmrB
SgrS
plac
GlmZ
RseX
GadY
MgrR
GlmY
OxyS
RyeB
MicC
MicA
RydC
ChiX
RybB
CyaR
GcvB
B.
PBAD-rpoS-lacZ
PBAD-rpoS-lacZ / PBAD-lacZ
Fold change compared to vector
10
5
2
1
-2
-5
-10
ChiX
DicF
CyaR
OxyS
RydC
MgrR
RyeB
GlmY
MicA
RybB
GcvB
GlmZ
GadY
RydD
plac
RseX
MicC
OmrB
MicF
OmrA
IS118
SgrS
Spot42
RyhB
RprA
DsrA
ArcZ

Fig_S3

Figure S4. A mutation outside the short form of ArcZ does not impair regulation of *rpoS*. A. The *rpoS* mRNA hairpin, as determined by (Soper and Woodson, 2008); the region involved in base-pairing with the sRNAs is shaded in grey. Nucleotides are numbered from the +1 of the *rpoS* mRNA. The AUG translation start codon is boxed, as is the nucleotide mutated in the experiment shown in panel C. The nucleotides mutated in the experiment in Fig. 2 are circled.

B. Predicted basepairing of each of three sRNAs with the shaded portion of the *rpoS* leader is shown below the hairpin. Mutated nucleotides are boxed and the changes made in DsrA, ArcZ, and the upper strand of *rpoS* are shown; positions are numbered according to the transcriptional start sites. Striped region is absent in the 55 nt processed form of ArcZ.

C. Cells containing either the *rpoS-lacZ* WT fusion (strain PM1409, left panel) or the *rpoS*(U468C)-*lacZ* mutant (strain PM1432, right panel) were assayed as for Fig. 2 for activation by plasmids containing either wild-type DsrA and ArcZ or the DsrA A11G or ArcZ A65G compensatory mutants (panel B). A parallel experiment done with the corresponding RprA mutant has been previously published (Majdalani et al, 2002).


A.
rpoS mRNA:
468
463
450
63nt
5'
3'
550
561
B.
ArcZ:
rpoS mRNA:
65
468
DsrA:
11
RprA:
C.
Specific activity
1200
1000
800
600
400
200
0
plac
DsrA
DsrA A11G
ArcZ
ArcZ A65G
rpoS WT
rpoS U468C

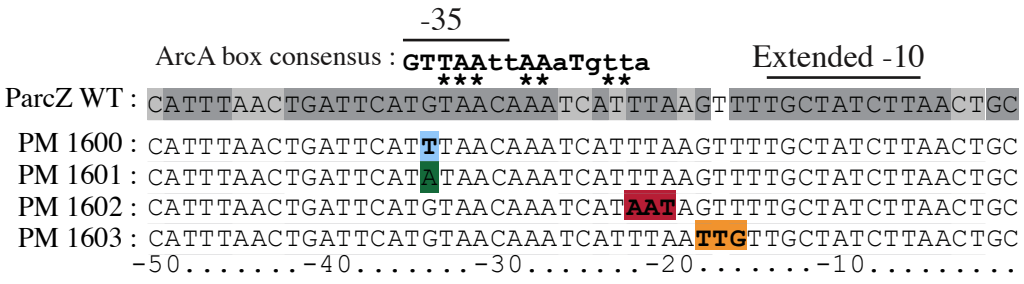
Fig_S4

Figure S5: *arcZ* Promoter Mutations.

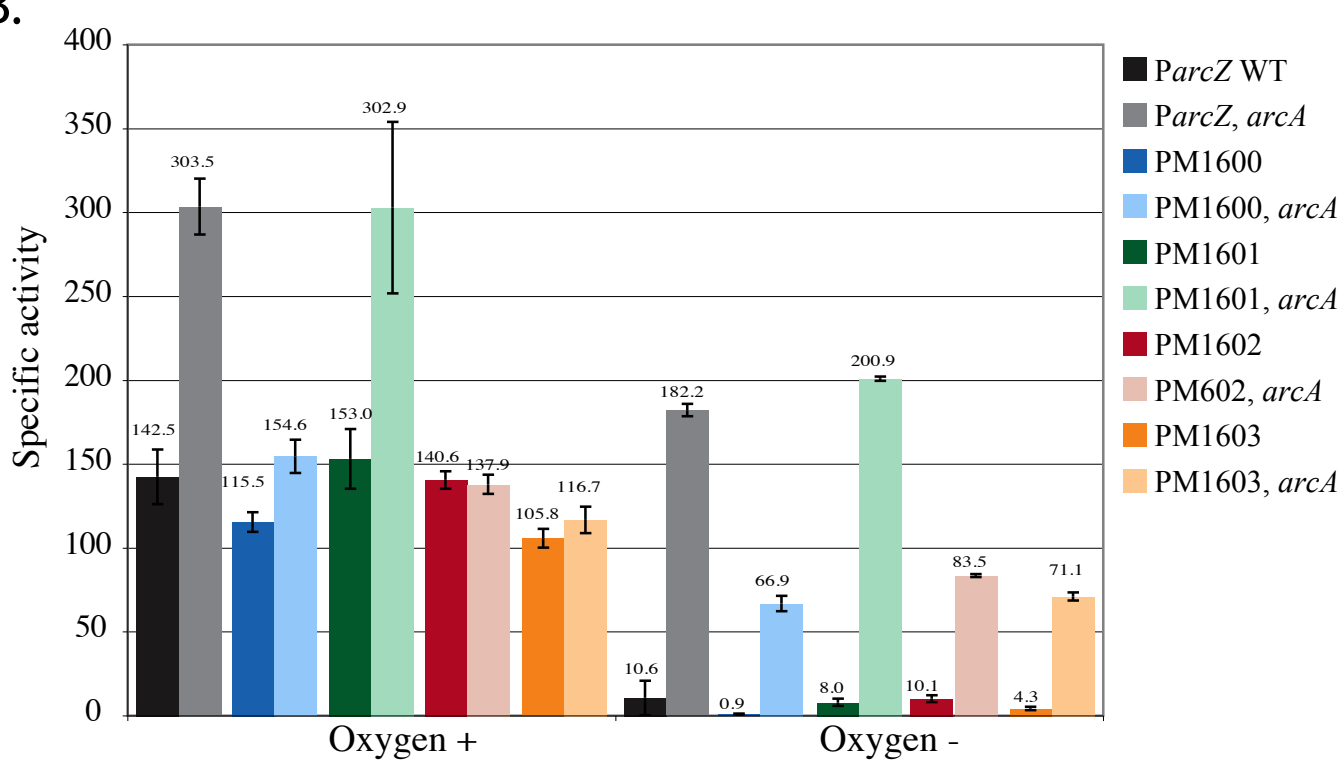
A. Point mutations used in the P*arcZ-lacZ* derivatives. The top sequence represents the WT sequence of the P*arc*Z promoter cloned into the P*arcZ-lacZ* fusion of strain PM1450, from -50 nt to +1. The transcription start site (+1) and the deduced extended -10 and -35 regions of the *arcZ* promoter are denoted by the text above the sequence. DNA sequence identities, as obtained by comparisons with other species (see fig. 4) are shaded in grey. The putative ArcA binding site overlapping the -35 element is indicated and residues that match the ArcA box consensus as defined by (McGuire et al., 1999) are indicated with stars. The mutations used in B are indicated with the nucleotide changes highlighted in corresponding to the graph. Strain names for the $arcA^+$ strains are to the left; *arcA*::*kan* derivatives are listed in Table S1.

B. Effect of mutations in the P*arcZ* promoter region. The WT P*arcZ*(-100)-*lacZ* strain (PM1450) and point mutant derivatives of the promoter region were grown in the presence or absence of oxygen to stationary phase (OD_{600} ≈1.5 and OD_{600}≈0.8 , respectively) . Numbers above each bar represent the measured specific activity. Each strain was also transduced with an *arcA*::*kan* mutation and assayed. Beta-galactosidase activity was measured as described in Material and Methods.

A.

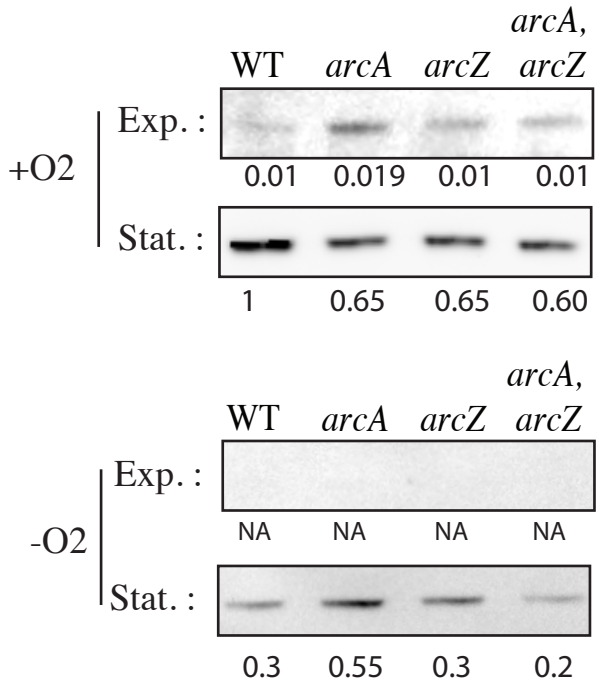

-35
ArcA box consensus : GTTAAttAAaTgtta
*** ** **
Extended -10
ParcZ WT : CATTTAACTGATTCATGTAACAAATCATTTAAGTTTTGCTATCTTAACTGC
PM 1600 : CATTTAACTGATTCATTTAACAAATCATTTAAGTTTTGCTATCTTAACTGC
PM 1601 : CATTTAACTGATTCATATAACAAATCATTTAAGTTTTGCTATCTTAACTGC
PM 1602 : CATTTAACTGATTCATGTAACAAATCATAATAGTTTTGCTATCTTAACTGC
PM 1603 : CATTTAACTGATTCATGTAACAAATCATTTAATTGTTGCTATCTTAACTGC
-50.....-40.....-30.....-20.....-10.....

B.


Specific activity
400
350
300
250
200
150
100
50
0
303.5
302.9
142.5
115.5
154.6
153.0
140.6
137.9
105.8
116.7
10.6
182.2
0.9
66.9
8.0
200.9
10.1
83.5
4.3
71.1
Oxygen +
Oxygen -
ParcZ WT
ParcZ, arcA
PM1600
PM1600, arcA
PM1601
PM1601, arcA
PM1602
PM602, arcA
PM1603
PM1603, arcA

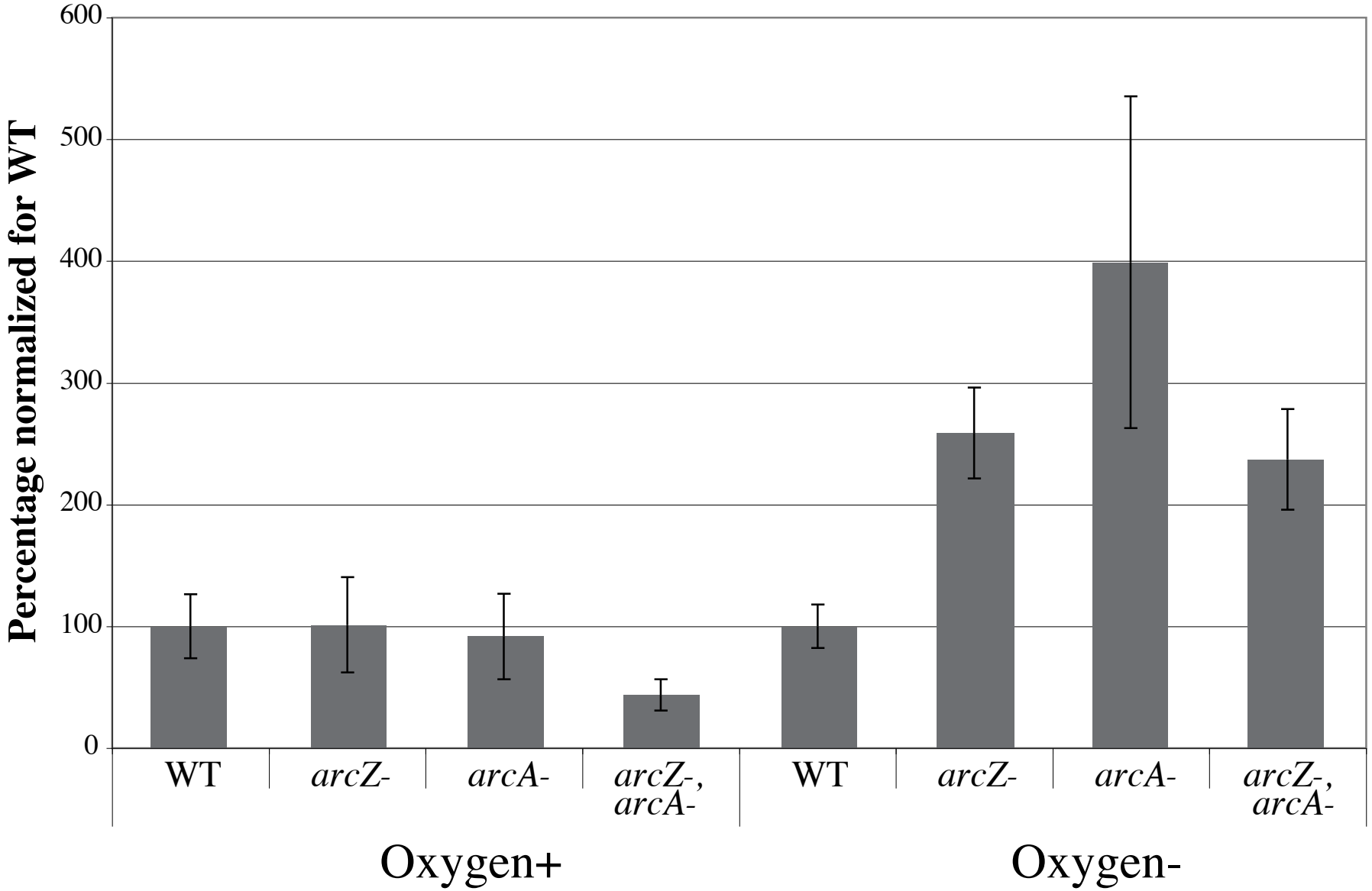
Fig_S5

Figure S6. Western blot of RpoS. Strain NM525 and its isogenic derivatives containing either an *arcA*::kan (PM1493) or a P*arcZ* KO (PM1520) mutation (see Fig. 6A), as well as a combination of both mutations (PM1534), were grown in LB at 37ºC in the presence or absence of oxygen. Samples were taken either at early exponential phase (Exp., OD_{600} = 0.1) or after 6 to 8 hours of growth (Stat.). Total proteins extracts were then prepared and resuspended into a volume of sodium dodecyl sulfate (SDS) sample buffer normalized for the OD_{600}, and used for western blotting experiments using an antibody directed against RpoS. The pictures shown come from one experiment, representative of three. Exposures and contrast were changed in order to allow proper visualization of the bands. Numbers below represent the densitometry of the bands that was quantified and normalized to 1 for the value of the WT sample in stationary phase in the presence of oxygen.


WT
arcA
arcZ
arcA, arcZ
+O2
Exp. :
0.01
0.019
0.01
0.01
Stat. :
1
0.65
0.65
0.60
-O2
Exp. :
NA
NA
NA
NA
Stat. :
0.3
0.55
0.3
0.2

Fig_S6

Figure S7: Real-Time RT-PCR analysis of *arcB* expression. Strain NM525 and its isogenic derivatives containing either an *arcA*::*kan* (PM1493) or a ParcZ KO (PM1520) mutation (see Fig. 6A), as well as a combination of both mutations (PM1534), were grown in LB at 37ºC in the presence or absence of oxygen up to stationary phase (OD600=1.5 and OD600=0.8, respectively). RNA was then extracted and submitted to real time RT-PCR as described in the supplementary Materials and Methods. Results of three experiments, each done in duplicate, are presented. Expression of *arcB* was normalized to SsrA levels and set to 100 for the WT strain in each condition.


Percentage normalized for WT
600
500
400
300
200
100
0
WT
arcZ-
arcA-
arcZ-, arcA-
WT
arcZ-
arcA-
arcZ-, arcA-
Oxygen+
Oxygen-

Fig_S7

Table S1- Strains used in this study

Strain Name	*Description*	*Reference*
MG1655	*E. coli* wild type	Laboratory strain
TKC	*tet, kan, cat* containing *E. coli* strain	(Sharan et al., 2009)
NM1100	MG1655 mini λ cm^R	(Bougdour et al., 2008)
NM525	MG1655 *lacI*^{q}	This study
PM1051	MG1655 *lacI*^{q}/p_{BAD}-*lacZ*	This study
PM1205	MG1655 *mal*::*lacI*^{q}, *ΔaraBAD, lacI'*:: P_{BAD}-*cat-sacB:lacZ, mini λ* tet^R	(Mandin and Gottesman, 2009)
KSJ6	*rprA1*::*kan* containing strain used for P1 transductions	(Majdalani et al., 2001)
DDS3201	*dsrA::cat* containing strain used for P1 transductions	(Sledjeski and Gottesman, 1995)
TX3077	*hfq1*::*cat* containing strain used for P1 transductions	(Tsui et al., 1997)
PM1409	PM1205 *lacI'*::P_{BAD}-*rpoS-lacZ*	This study
PM1411	PM1409 *dsrA::cat*	This study
PM1412	PM1409 *rprA::kan*	This study
PM1413	PM1409 *arcZ::tet*	This study
PM1414	PM1409 *dsrA::cat, rprA::kan*	This study
PM1415	PM1409 *dsrA::cat, arcZ::tet*	This study
PM1416	PM1409 *rprA::kan, arcZ::tet*	This study
PM1417	PM1409 *dsrA::cat, rprA::kan, arcZ::tet*	This study
PM1419	PM1409 *hfq::cat*	This study
PM1432	PM1205 *lacI'*::P_{BAD}-*rpoS(U468C)-lacZ*	This study
PM1433	PM1205 *lacI'*::P_{BAD}-*rpoS(G463C)-lacZ*	This study
PM1438	PM1205 *lacI'*::P_{BAD}-*rpoS(C561G)-lacZ*	This study
PM1439	PM1205 *lacI'*::P_{BAD}-*rpoS(G463C-C561G)-lacZ*	This study
PM1480	PM1409 *arcA::kan*	This study
PM1481	PM1480 *arcZ::tet*	This study
PM1450	PM1205 *lacI'*::P*arcZ*(-100)-*lacZ*	This study
PM1451	PM1205 *lacI'*::P*arcZ*(-100)(TG-14/-15CC)-*lacZ*	This study
PM1453	PM1450 *arcA::kan*	This study
PM1456	PM1450 *arcB::kan*	This study
PM1051	PM1205 P_{BAD}-*lacZ*	This study
PM1493	NM525 *arcA::kan*	This study
PM1520	NM525 P*arcZ* KO	This study
PM1534	PM1520 *arcA::kan*	This study
PM1560	NM525 ΔP*arcB*::*cat*-PBAD-*arcB*	This study
PM1561	PM1560 P*arcZ* KO	This study
PM1562	PM1560 *arcA*::kan	This study
PM1563	PM1561 *arcA*::kan	This study
PM1600	ParcZ(G-35T)-*lacZ*	This study
PM1601	ParcZ(G-35A)-*lacZ*	This study
PM1602	ParcZ(TTA-23AAT)-*lacZ*	This study
PM1603	ParcZ(GTT-19TTG)-*lacZ*	This study
PM1604	PM1600 *arcA::kan*	This study
PM1605	PM1601 *arcA::kan*	This study
PM1606	PM1602 *arcA::kan*	This study
PM1607	PM1603 *arcA::kan*	This study

SG30013	DJ480 (*rpoS750*::*lacZ* translational fusion)	Wassarman et al., 2001
PM1620	SG30013 *arcA::kan*	This study
PM1621	SG30023 *arcZ*::*tet*	This study
PM1622	SG30013 *arcA::kan, arcZ*::*tet*	This study

Table S2. Oligonucleotides and probes used in this study.

Oligos Name	Sequence (5' to 3')
	Oligonucleotides used for cloning into the sRNA library
AatII-RydC-for	GCGTGACGTCCTTCCGATGTAGACCCGTATTC
EcoRI-RydC-rev	GCGAGAATTCAAGGATATTTAaagAAAACGCC
AatII-RprA-for	GCGTGACGTCacgGTTATAAATCAACATATTGATTA
EcoRI-RprA-rev	GCGAGAATTCGAAAGAGTGAGGGGCGAGGTAGC
AatII-RyhA-for	GCGTGACGTCgtgCGGCCTGAAAAACAGTGC
EcoRI-RyhA-rev	GCGAGAATTCGTAGAAGTGCTGAAAGCGTGG
AatII-GlmZ-for	GCGTGACGTCgtaGATGCTCATTCCATCTC
EcoRI-GlmZ-rev	GCGAGAATTCCCTCCGGGGCCTTCCTGATAC
AatII-GlmY-for	GCGTGACGTCAGTGGCTCATTCACCGACTTATG
EcoRI-GlmY-rev	GCGAGAATTCGTGTTACTCTCGTCAGACGCG
AatII-OxyS-for	GCGTGACGTCGAAACGGAGCGGCACCTCTTTTAACCC
EcoRI-OxyS-rev	GCGAGAATTCCGCCGGGCTTTTTTATGGCA
AatII-DicF-for	GCGTGACGTCtttCTGGTGACGTTTGGCGG
EcorI-DicF-rev	GCGAGAATTCGCGCTCAGCCGCATTCACCACA
AatII-Spot42-for	GCGTGACGTCgtaGGGTACAGAGGTAAGATGTTCTATC
EcorI-Spot42-rev	GCGAGAATTCGCGCATCAGGCATTACGGATC
AatII-resX-for	GCGTGACGTCtttTTATTATTCTGTGTCATGATGC
EcorI-rseX-rev	GCGAGAATTCACATAGGGGGTATCAATGATTGTTG
AatII-FnrS-for	GCGTGACGTCGCAGGTGAATGCAACGTCAAGCGATG
EcorI-FnrS-rev	GCGAGAATTCCTGGAACAGGATCGCCAGGAATC
AatII-DsrA	GGCCAAGACGTCAACACATCAGATTTCCTGGTGTAACG
DsrA-Eco	GCAGCAGAATTCAAAAAAATCCCGACCCTGAGGG
AatII-RyhB	GGCCAAGACGTCGCGATCAGGAAGACCCTCGCGGAG
RyhB-Eco	GCAGCAGAATTCAAAAAAAAAGCCAGCACCCGGCTGGC
AatII-MicC	CGATGACGTCGTTATATGCCTTTATTGTCACAG
MicC-Eco	CGTACGAATTCGGATAAGGATTATCCAATTCTAAA
AatII-MicF	CGATGACGTCGCTATCATCATTAACTTTATTTAT
MicF-Eco	CGTACGAATTCAGTGTGTAAAGAAGGGTAAAAAA
AatII-GcvB	CGATGACGTCACTTCCTGAGCCGGAACGAAAAG
GcvB-Eco	CGTACGAATTCGTAATTCGCGATCGCAAGGTAA
	Oligonucleotides used for mutant constructions
arcB-kan-for	CGCATTTCTCACACAATTTATAACGTAACTGTCAGAATTGAAAGCCACG
arcB-kan-rev	TTGTGTCTCAA
arcA-kan-for	CCCAGCTGTTGCAGATGGCGTAACCCCACTGACCCCGCCGGCGCTGAGG
arcA-kan-rev	TCTGCCTCGTG
arcZ-tet-for	TTTAGGTAGCAAACATGCAGACCCCGCACATTCTTATCGTTATGGACAG
arcZ-tet-rev	CAAGCGAACCG
arcZ-cat-for	CCGTTTTTTTTGACGGTGGTAAAGCCGAttaATCTTCCAGCAGAAGAACTC

arcZ-sac-rev	GTCAAGAAG
ParcZ KO	CACATTTAACTGATTCATGTAACAAATCATTTAAGTTTTGTCCTAATTTTT TTGACACT GTAGAAGTGCTGAAAGCGTGGGTGGCAAAAGCCACTAAAACTCTTGGG TTATCAAGAGGG
arcZ-5'-RACE1	ACACATTTAACTGATTCATGTAACAAATCATTTAAGTTTTAATGAGACG
arcB-3'RACE	TTGATCGGCAC
PBAD-RNA	TTACAAGGGCACAGCACTGTTTTTCAGGCCGcacGCAGTTACTGTCCATA
PBAD-DNA	TGCACAGATG
M13-for	ACACATTTAACTGATTCATGTAACAAATCATTTAAGTTTTGCCCCCCCTA
M13-rev	ACTGCgtgCGGCCTGAAAAACAGTGCTGTGCCCTTGTAA
	Oligonucleotides for 5'- and 3'-RACE
	CTAGACCGGGGTGCGCG
PBAD-rpoSP-for	CTGGGAAGATAACGTCGGTGAATG
lacZ-rpoS-rev	acUcUcUacUgUUUcUccaU
RpoSB2-for	accTgacgcTTTTTaTcgcaacTcTcTacTgTTTcTccaT
RpoSB2-rev	CAGGAAACACGTATGAC
rpoS(G463C)-for	GTAAAACGACGGCCAG
rpoS(G463C)-rev	
rpoS(C561G)-for	Oligonucleotides used lacZ fusions constructions
rpoS(C561G)-rev	
PryhA-100-F	acctgacgctttttatcgcaactctctactgtttctccatCGGGTGAACAGAGTGCTAAC
PryhA-+1-R	taacgccagggttttcccagtcacgacgttgtaaaacgacATCATGAACTTTCAGCGTAT GGGGAAACCCGTAAACCCGCTGCGTTAT
PryhA-+1-mut-R	TTTACGGGTTTCCCCTTGTAACGAATTT TACAAGGCGAAATCCGTAAACCCGCTGC
5'PBAD	GGATTTCCCCTTGTAACGAATTTCAAAA
deeplac	TAGGAGCGACCTTATGAGTCAGAATACG
PBAD-lacZ	ATAAGGTCGCTCCTACCCGTGATCCCTT CGAAGCGGCATGCATTTACGTTGACACCATCGAATGGCGCCGCAATTGA CTGAAACACAT taacgccagggttttcccagtcacgacgttgtaaaacgacCATAGCTGTTTCCTGTGTGACGCA GTTAAGATAGCAAAAC
DsrA(A11G)-for	taacgccagggttttcccagtcacgacgttgtaaaacgacCATAGCTGTTTCCTGTGTGACGCA
DsrA(A11G)-rev	GTTAAGATAGGAAAAC
RprA(A37G)-for	cgacgaattcgcgcttcagccatactttcatac
RprA(A37G)-rev	CGGGCCTCTTCGCTA
ArcZ(A56G)-for	ACCTGAGCGTTTTTATCGCAACTCTCTACTGTTTCTCCATAATTGTGAGC
ArcZ(A56G)-rev	GGATAACAATTCACACAGGAAACAGCTATGGTCGTTTTACAACGTCGTG
DsrA(C16G)-for	ACTGGGAAAACAATGGCGTT
DsrA(C16G)-rev	
RprA(C42G)-for	Oligonucleotides used for Quickchange mutagenesis:
RprA(C42G)-rev	
ArcZ(C70G)-for	cgtcacgAACACATCAGGTTTCCTGGTGTAACGAA
ArcZ(C70G)-rev	TTCGTTACACCAGGAAACCTGATGTGTTcgtgacg

	ATATTGATTTATAAGCATGGGAATCCCCTGAGTGAAACAAC
	GTTGTTTCACTCAGGGGATTCCCATGCTTATAAATCAATAT
arcBqPCR for	CGGCGCAGCCAAGGTTTCCCTGGTGTT
arcB-qPCR-rev	AACACCAGGGAAACCTTGGCTGCGCCG
qssrA-F	cacgAACACATCAGATTTCGTGGTGTAACGAATTTTTTA
qssrA-R	TAAAAAATTCGTTACACCACGAAATCTGATGTGTTcgtg
	GATTTATAAGCATGGAAATCGCCTGAGTGAAACAACGAATT
	AATTCGTTGTTTCACTCAGGCGATTTCCATGCTTATAAATC
SgrS-NB1 (SgrS1-bio)	CAGCCAAGATTTCGCTGGTGTTGGCGC
	GCGCCAACACCAGCGAAATCTTGGCTG
RydC NB1	
ArcZ NB1	Oligonucleotides for Real-Time RT-PCR:
ArcZ NB4	TCGCGACATTACCGAGCGTA
GlmZ NB1	AGCAGAATGCGGCTCAGACC
GlmY NB1	TTTGTTAGTGGCGTGTCCGT
RseX NB1	GAACCCGCGTCCGAAAT
FnrS-NB1	Biotinylated probes used for Northern blots
GcvB NB1	
RybD NB1	GCAACCAGCACAACTTCGCTGTCGCGGTAAAATAGTG
MicA NB1	CTAAAACCGACCCGTGGTACAGGCGAAGAATACGGGTCT
RyeB NB1	CGCCGTAAATTATTATGATGAGTTACAAGGGCACAGCAC
RybC NB	GGCTAGACCGGGGTGCGCGAATACTGCGCCAACACCAGGG
IS118 NB1	GTGGACGATAAGCACCGTAAACGGCTCTGCGTCATTCCGG
MgrR NB1	CATTCGTATTTTATGTAGCACGTCCCGAAGGGGCTG
OxyS NB1	GATAAAAGGCTAATAACGGAAGCATCATGACACAG
RybB NB1 (KT98)	GGAAGTAAGACAATATGGAGCGCAACGCCCATCGC
RprA NB1	CCAGAACACGCATTCCGATAAAACTTTTCGTTCCGGCTCAGG
CyaR	GGCCAACCATGTCGAAACCGACAGCCCTTCGCC
NB1(ryeEProbe1)	CATCTCTGAATTCAGGGATGATGATAACAAATGCGCGTCTtt
OmrA probe1	CGGTCCAGGGAAATGGCTCTTGGGAGAGAGCCGTGCGC
OmrB probe1	CATTTTTTTATTATTATGCCGTCACTTTAAGCGACGGTG
RyhB NB1 (EM1)	CCAATCATGGCGCGCACAAGCTATAATACCAAC
GadY NB1	CAGTAAACCGGCGGTGAATGCTTGCATGGATAGAT
DicF NB1	AAACTCTCGAAACGGGCAGTGACTTCAAGGGTTAAA
Spot42 NB1	CAAAATGGGGACATCAAAGAAAAGCAGTGGC
SsrA	GGGGATTTC CATGCTTATAAATCAATATGT
ArcB NB2	TGGTTCCTGGTACAGCTAGCATTTTATGGGTTATG
	GAGACAGGGTACGAAGAGCGTACCGAATAATCTCACC
	CATGTGCTCAACCCGAAGTTGACTTCACCTATCAATACC
	AAGTAATACTGGAAGCAATGTGAGCAATGTCGTGCTTTCAGGTTCTC
	GGGGACCGGGAGAGGATAGTCTGCCGTCTCCAGAC
	GGCAGAGCAGTCACGGAGTAAAACTGATACCGCC
	GAAGTAAAAGGTCTGAAAGATAGAACATCTTACCTC
	CGCCACTAACAAACTAGCCTGATTAAGTTTTAACGCTTCA
	CGGCCAGACCAATACCGGTGCCGGTGGCAGGTTTACCG

Supplementary Material and Methods:

Strain construction

The *arcA::kan*, *arcB::kan* and the *arcZ*::*tet* mutants were obtained by PCR amplifying the *ka*n and the *tet* cassettes contained in the TKC strain (Sharan et al., 2009), respectively, with the oligonucleotides listed in Table S2, using the Expand High Fidelity PCR system (Roche). The PCR product was then purified and recombined into strain NM1100 by λ red recombinase-mediated gene replacement (Court et al., 2003). Marked mutations were moved into the desired strain backgrounds using bacteriophage P1 transduction as described previously (Silhavy et al., 1984). PCR amplifications were carried out using the Expand High Fidelity PCR system (Roche).

The P_{BAD}-*rpoS-lacZ* and the P*arcZ-lacZ* fusions were constructed by λ red-mediated recombination in strain PM1205, as previously described (Mandin and Gottesman, 2009a). Briefly, a PCR fragment corresponding to the *rpoS* leader or to the P*arcZ* promoter were amplified by PCR with oligonucleotides PBAD-rpoSP-for and lacZ-rpoS –rev (to create PM1409), or PryhA-100-F and PryhA-+1-R (to create PM1450) respectively. Mutations in the *rpoS-lacZ* fusion used to demonstrate specificity of interaction with the sRNAs were constructed by amplifying the *rpoS-lacZ* sequence from PM1409 with primers rpoS(G463C)-for, rpoS(G463C)-rev, rpoS(C561G)-for, rpoS(C561G)-rev, and PBAD and deeplac by overlap PCR. The PCR products were then inserted into the chromosome of strain PM1205 by λ red-mediated recombination. The resulting PCR fragments were then recombined in the chromosome of strain PM1205 to generate the final strains. The P_{BAD}-*lacZ* fusion was obtained by recombining the PBAD-lacZ oligonucleotide directly into strain PM1205.

The *rpoS*(U468C)-*lacZ* containing strain was constructed by amplifying the *rpoS-lacZ* sequence of strain PM1409 with primers rpoS-B2-for, rpoSB2-rev and 5'PBAD and deeplac by overlap PCR. Mutations in the ParcZ-lacZ fusion were obtained by directly amplifiying the ParcZ-lacZ sequence from strain PM 1450 with oligonucleotides ParcZ.arca1a(G-35T)-for (5'-CTGATTCATtTAACAAATCATTTAAGTTTTG-3'), ParcZ.arca1a(G-35T)-Rev (5'-atttgtTAaATGAATCAGTTAAATGTGTG-3"), ParcZ.arca1B(G-35A)-for (5'-CTGATTCATaTAACAAATCATTTAAGTTTTG-3'),

ParcZ.arca1b(G-35a)-rev (5'-aTTTgtTAtATGAATCAGTTAAATGTGTG-3'), ParcZ.arca1C(tta-23aat)-for (5'-CAAATCATaatAGTTTTGCTATCTTAACTGCg-3'), ParcZ.arca1C(tta-23aat)-rev (5'-gcaaaACTattATGATTTGTTACATGA-3'), Parc.arca2a(gtt-19ttg)-for (5'-CATTTAAttgTTGCTATCTTAACTGCg-3') and Parc.arca2a(gtt-19ttg)-rev (5'-GATAGCAAcaaTTAAATGATTTGTTACATG-3') with oligonucleotide deep-lac (for forward primers) or PryhA-100F (for reverse primers) by overlap extension PCR. The obtained PCR product were then directly cloned into strain PM1205, as described previously (Mandin and Gottesman, 2009a).

Library screening

Plasmids were introduced into the appropriate strains by TSS transformation with slight modification (Chung and Miller, 1988). Briefly, cells were grown in 30 mL of LB medium to an OD_{600} of 0.5, centrifuged and resuspended in 3mL of cold TSS solution (LB, 5% DMSO, 50mM $MgSO_4$, 10% PEG8000). 100μL per well of resuspended cells were then dispensed into wells of microtiter plates and 1μL of each plasmid from the library was added. After 30 minutes on ice, the microtiter plate was incubated at 37ºC with agitation for 1 hour and 4μL of cells were then spotted on LB ampicillin-containing plates for selection of transformants. Colonies were then used to inoculate LB medium containing ampicillin (100 μg/mL), arabinose and IPTG (100 μM) at 37º C for 6h in microtiter plates with medium agitation, then lysed. An arabinose concentration of 0.002% yielded a basal activity for the PM1409 (WT P_{BAD}-*rpoS-lacZ* fusion with the pBR-plac vector of 138 specific units (approximately 600 Miller Units), allowing both positive and negative effects to be easily detected. For the other strains, levels of arabinose were adjusted to the specific fusion and expression conditions. β-galactosidase activity was then measured in microtiter plates as described previously (Majdalani et al., 1998).

Western blotting experiments

Whole cell extracts were prepared as follows: 1mL of cell culture at the appropriate OD_{600} was centrifuged and the pellet was resuspended into a volume of dodecyl sulfate (SDS) sample buffer normalized for the OD_{600} and boiled for 10 minutes

at 95ºC. Electrophoresis migration of the samples was performed on NuPAGE 12% Bis-Tris gels (Invitrogen), transferred and probed with a 1:5000 dilution of RpoS antiserum. Blots were then developed using the Lumi-Phos WB chemiluminescent system (Thermo scientific).

Real Time RT-PCR experiments

RNA for real time RT-PCR experiments was extracted from the appropriate strains from cultures grown in LB up to stationary phase (OD_{600}=1.5) in aerobic conditions, or after overnight growth in anaerobic conditions. RNA was DNase treated twice (Turbo DNase) after preparation. 1μg of purified RNA was then used to generate cDNA using random hexamers and the SuperScript™ First-Strand Synthesis System (Invitrogen), according to manufacturers instructions. Real-Time PCR were done using the SYBR Green Supermix. Reactions were done in a 96-well microtiter PCR plate using 5 µl of cDNA and final concentration 0.2 µM sense and antisense primers for amplifying SsrA and the *arcB*-mRNA (arcBqPCR for, arcB-qPCR-rev, qssrA-F, and qssrA-R, Tablse S2). Cycling conditions were as follows: denaturation (95°C for 3 min), amplification and quantification (95°C for 30 s, 55°C for 25 s and 72°C for 30 s, with a single fluorescence measurement at both 55°C and 72°C for 30 s segments) repeated 40 times, a melting curve program (55–95°C with a heating rate of 0.1°C s^{-1} and continuous fluorescence measurement), and a cooling step to 50°C. Data was analyzed using the iQcycler analysis software (Bio-Rad). The expression level of *arcB* was normalized by the level of the internal control SsrA.

Supplementary references

Bougdour, A., Cunning, C., Baptiste, P.J., Elliott, T. and Gottesman, S. (2008) Multiple pathways for regulation of σ^S (RpoS) stability in *Escherichia coli* via the action of multiple anti-adaptors. Mol Microbiol, 68, 298-313.

Chung, C.T. and Miller, R.H. (1988) A rapid and convenient method for the preparation and storage of competent bacterial cells. Nucleic Acids Res, 16, 3580.

Court, D.L., Swaminathan, S., Yu, D., Wilson, H., Baker, T., Bubunenko, M., Sawitzke, J. and Sharan, S.K. (2003) Mini-lambda: a tractable system for chromosome and BAC engineering. Gene, 315, 63-69.

Majdalani, N., Chen, S., Murrow, J., St John, K. and Gottesman, S. (2001) Regulation of RpoS by a novel small RNA: the characterization of RprA. Mol Microbiol, 39, 1382-1394.

Majdalani, N., Cunning, C., Sledjeski, D., Elliott, T. and Gottesman, S. (1998) DsrA RNA regulates translation of RpoS message by an anti-antisense mechanism, independent of its action as an antisilencer of transcription. Proc Natl Acad Sci U S A, 95, 12462-12467.

Majdalani, N. Hernandez, D. and Gottesman, S. (2002) Regulation and mode of action of the second small RNA activator of RpoS translation, RprA. Mol Microbiol, 46, 813-826.

Mandin, P. and Gottesman, S. (2009) A genetic approach for finding small RNAs regulators of genes of interest identifies RybC as regulating the DpiA/DpiB two-component system. Mol Microbiol, 72, 551-565.

McGuire, A.M., De Wulf, P., Church, G.M. and Lin, E.C. (1999) A weight matrix for binding recognition by the redox-response regulator ArcA-P of *Escherichia coli*. Mol Microbiol, 32, 219-221.

Sharan, S.K., Thomason, L.C., Kuznetsov, S.G. and Court, D.L. (2009) Recombineering: a homologous recombination-based method of genetic engineering. Nat Protoc, 4, 206-223.

Silhavy, T., Berman, M. and Enquist, L. (1984) Experiments with gene fusions. Cold Spring Harbor Laboratory.

Sledjeski, D. and Gottesman, S. (1995) A small RNA acts as an antisilencer of the H-NS-silenced *rcsA* gene of *Escherichia coli*. Proc Natl Acad Sci U S A, 92, 2003-2007.

Soper, T.J. and Woodson, S.A. (2008) The *rpoS* mRNA leader recruits Hfq to facilitate annealing with DsrA sRNA. Rna, 14, 1907-1917.

Tsui, H.C., Feng, G. and Winkler, M.E. (1997) Negative regulation of *mutS* and *mutH* repair gene expression by the Hfq and RpoS global regulators of *Escherichia coli* K-12. J Bacteriol, 179, 7476-7487.

Wassarman, K.M., Repoila, F., Rosenow, C., Storz, G. and Gottesman, S. (2001) Identification of novel small RNAs using comparative genomics and microarrays. Genes Dev, 15, 1637-1651.